

**DECLARATION
AND POWER OF ATTORNEY
Original Application**

As a below named inventor, I declare that the information given herein is true, that I believe that I am the original, first and sole inventor if only one name is listed at 1 below, or a joint inventor if plural inventors are named below, of the invention entitled:

**LOW DOSE HAPTENIZED TUMOR CELL AND
TUMOR CELL EXTRACT IMMUNOTHERAPY**

which is described and claimed in:

☒ the attached specification or ☐ the specification in application
Serial No. , filed
(for declaration not accompanying appl.)

that I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to this application, or in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application, that I acknowledge my duty to disclose information of which I am aware which is material to patentability in accordance with 37 CFR §1.56. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I hereby claim the priority benefits under 35 U.S.C. §119 of any application(s) for patent or inventor's certificate listed below. All foreign applications for patent or inventor's certificate on this invention filed by me or my legal representatives or assigns prior to the application(s) of which priority is claimed are also identified below.

PRIOR APPLICATION(S), IF ANY, OF WHICH PRIORITY IS CLAIMED

<u>COUNTRY</u>	<u>APPLICATION NO.</u>	<u>DATE OF FILING</u>
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LAST NAME: FIRST NAME: MIDDLE NAME:

CITY: STATE OR FOREIGN COUNTRY: COUNTRY OF CITIZENSHIP:

POST OFFICE ADDRESS: CITY: STATE OR COUNTRY: ZIP CODE:

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 1: _____ DATED: _____
David BERD

SIGNATURE OF INVENTOR 2: _____ DATED: _____

SIGNATURE OF INVENTOR 3: _____ DATED: _____

*1568

Antitumor effects in patients with melanoma, head and neck and breast cancer in a phase III clinical trial of Interleukin-12 (IL-12) gene therapy. Hideaki Tahara, Laurence Zitvogel, Walter J. Storkus, Elaine M. Elder, Donna Kinzler, Theresa L. Whiteside, Paul D. Robbins, and Michael T. Lotze. Departments of Surgery, Molecular Genetics and Biochemistry, University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA.

IL-12 is a heterodimeric cytokine which induces high-level production of IFN-gamma, promotes Th1 type responses and has potent antiangiogenic effects. Injection of the fibroblasts expressing IL-12 genes into murine tumors can eradicate established tumor and elicit systemic anti-tumor immune reaction specific for the tumor at a distant site (Cancer Research 54: 1994; J. Immunol. 154: 6466, 1995). We initiated a Phase I/II clinical trial of IL-12 gene therapy using direct injection of tumors with genetically engineered autologous fibroblasts based on these promising results. Fibroblast cultures were successfully established from the patients' dermal skin, transduced with a retroviral vector expressing human IL-12 (TFG-hIL-12-Neo) and selected with G418. High expression of heterodimeric IL-12 protein from the transduced fibroblasts was observed after selection (usually over $150\text{--}200/10^6$ cells/24 hours). This protocol was initiated in July of 1995, and the first eighteen patients have been treated with weekly injections of fibroblasts designed to deliver 10, 30, 100, 300, 100 or 300 ng/24 hours in cohorts of 3 patients. 7 breast cancer, 1 thyroid cancer, two head and neck cancer, one colorectal cancer, and 7 melanoma patients have been treated. No untoward effects have been observed. Three patients with recurrent melanoma, one with head and neck carcinoma, one with thyroid carcinoma have been observed to have shrinkage of the injected lesions as well as distant lesions by more than 50%.

*1569

MUC-1 keyhole limpet hemocyanin (KLH) conjugate plus QS-21 vaccination of high risk breast cancer patients (BCPTs) with no evidence of disease (NED). T. Gilewski, R. Adluri, S. Zhang, A. Houghton, L. Norton, P. Livingston. Memorial Sloan-Kettering Cancer Center, New York, NY.

Stage IV NED BCPTs or earlier stage BCPTs NED except for rising CEA or BR2729 levels are at high risk for overt recurrence and might benefit from immunotherapy. Mucin MUC-1, found on most breast carcinomas, is a potential target. A synthetic 30 amino acid (aa) sequence of MUC-1 has been conjugated with KLH and mixed with the immune adjuvant QS21 to increase immune recognition. Nine pts (ages 43-61 years) have been vaccinated: 8 stage IV NED, 1 stage II with increased CEA level and NED, all but one stage IV NED pt on hormonal tx. All pts received 5 doses of 100 mcg MUC-1 s.c. given on weeks 1, 2, 3, 7, 19. All pts had transient grade 2 local toxicity at the vaccine site and most had grade 1-2 flu like symptoms. All pts remain NED (median follow up 55 weeks) although one pt had a chest wall recurrence which was excised. For all pts the range of IgM and IgG reciprocal titers against purified MUC-1 by ELISA are:

Week #	0	3	5	13	21
IgM	0-160	10-20,480	1280-20,480	10-20,480	320-20,480
IgG	0-10	0-320	40-20,480	160-2560	640-10,240

Five pts maintain IgG titers (range 320-1280) between 6-12 months following the last vaccine. Analysis of IgG subclasses in 8 pts reveal predominantly IgG1 and IgG3. Immune adherence rosetting against MCF-7 cell lines revealed an increase in IgM titers in 6/7 pts. Inhibition assays demonstrate that all sera react exclusively with the APDTRPA determinant of MUC-1. No evidence for augmentation of T cell immunity was found. This MUC-1 vaccine is immunogenic in breast cancer pts who are NED. (Support from NCI PO1 CA33049)

1570

Delayed-type hypersensitivity (DTH) responses induced by autologous, hapten-modified melanoma vaccine - Importance of dosage schedule. D. Berd, M.J. Mastrangelo, E. Bloome, W. Medley, C. Clarke, and H.C. Maguire, Jr. Thomas Jefferson University, Philadelphia PA.

We have reported that administration of a vaccine consisting of autologous melanoma cells modified with the hapten, dinitrophenyl (DNP-vaccine), prolonged relapse-free and overall survival in patients with clinical stage 3 melanoma following lymphadenectomy. We have compared four dosage-schedules of DNP-vaccine in post-surgical adjuvant patients to determine which are more effective in inducing DTH to autologous, unmodified melanoma cells (autol-MEL): A=q 28 days x8, all vaccines DNP-modified; B=weekly x 12, alternating DNP-modified and unmodified vaccine; C=weekly x12, all vaccines DNP-modified; and D=weekly x6, all vaccines DNP-modified. Patients on all schedules except D were sensitized to the hapten prior to vaccine. In all four regimens BCG was mixed with the melanoma cells to provide an immunological adjuvant. Dosage-schedules A and D induced significantly greater DTH to autol-MEL than the more intensive schedules, B and C ($p=.001$, Mann-Whitney U test). The proportion of patients who developed a DTH response to autol-MEL $\geq 5\text{mm}$ was as follows: A=20/44=45%, B=3/27=11%, C=4/22=18%, D=16/27=59% ($p<.01$, Chi square). In contrast, all four dosage-schedules induced similar DTH responses to PPD. Follow-up to date suggests that the two dosage-schedules (A and D) that were most effective in inducing DTH to autol-MEL produced longer relapse-free survivals than the two schedules (B and C) that were the least immunologically effective, even after adjusting for standard prognostic variables. Thus, the dose and schedule of administration of human tumor vaccines may be as critical as their composition in inducing immunological responses that have clinical meaning.

1571

A phase I trial of bispecific antibody (BsAB) MDX447 without and with granulocyte colony-stimulating factor (G-CSF) in patients with adult solid tumors. R.T. Cymow, D.G. Pfister, Y. Deo, R.J. Motzer, C. Winston, K.L. Keeperman, T. Malone. Memorial Sloan-Kettering Cancer Center, New York, NY; Medarex, Inc., Annandale, NJ.

MDX447 is a BsAB constructed by cross-linking F(ab') fragments of monoclonal antibody (MoAB) H22 to FcγRI and MoAB H425 to the epidermal growth factor receptor (EGFR). In vitro, MDX447 effects lysis of EGFR overexpressing cell lines; FcγRI-positive neutrophils (PMNs) constitute a major effector cell population during G-CSF therapy. We have enrolled 26 patients (pts) (median age 54 [38-78]; median Karnofsky 85 [70-90]; male/female 17/9; prior systemic therapy 25: primary cancer kidney 11, head & neck 8, bladder 2, other 3) in a phase I study evaluating MDX447 +/- G-CSF. Successive groups of 3-6 pts received MDX447 intravenously (IV) weekly (days 1,8,15,22,29,etc.) alone, or with G-CSF (3 mcg/kg/day) subcutaneously (SQ) (days -3 to 1, 4-8, 11-15, etc.). Dose levels of MDX447 evaluated thus far include 1 and 3.5 mg/m²; 7 mg/m² is in progress. Primary toxicities encountered include fever, chills, blood pressure lability, pain/myalgias, and grade 2 increase in 5'nucleotidase; most toxicities abated within 12 hours. Maximum tolerated and biologic dose have yet to be defined. G-CSF induced upregulation of FcγRI on PMNs; in vivo binding of MDX447 to monocytes occurred in all pts, but to a significant degree to the PMNs of only G-CSF treated pts. Of 22 pts evaluable for response, there have been no major responses; 15 pts had stable disease beyond the first month of therapy. Dose escalation continues to better define the dose, toxicity, and potential therapeutic role of this novel biologic.

PROGRAM/PROCEEDINGS
AMERICAN
SOCIETY OF
CLINICAL
ONCOLOGY

Thirty-Third Annual Meeting

May 17-20, 1997
Denver, CO